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ON DIFFERENTIATION OF AMPULLA OF LARVA OF AN  
ASCIDIAN, *HALOCYNTHIA RORETZI*<sup>1)</sup>

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In *Halocynthia roretzi*, when the larvae soon after hatching are kept at 3–5°C, one third of those larvae kept their swimming activity for 23 days without absorption of their tails, which they destroyed later (Hirai, 1962 and 1963). In those larvae, it is supposed that the metamorphosis or absorption of the tail and metamorphosis of the body can take place separately due to the effect of the lower temperature. When the larvae with the metamorphosing bodies and the unabsorbed tails are treated by the solution of Nile Blue, the disturbed processes of both metamorphoses of the tail and the body are awakened again to proceed further in their differentiations of metamorphosis. In this case, the adhesive papillae are either visible or have not disappeared due to the effect of the lower temperature. In 1964, the present writer observed an other fact on the differentiation of the ampulla in the experiment of the effect of the lower temperature.

MATERIAL AND METHOD

*Halocynthia roretzi* which was used in the present experiment was collected from off Asamushi in November 1964. The larvae were obtained from the naturally spawned eggs in the laboratory.

OBSERVATION

When the fertilized eggs of this species are kept at about 10°C, the larvae hatched out at about 24 hours after the fertilization. In the present experiment, the larvae soon after hatching were kept at about 3–5°C in an ice-box. In the larvae that kept swimming without absorption of the tails in the treatment by lower temperature for eight days, two types of differentiations of the body were observed.

One of them was the same as the type observed in the previous experiment, and the body was metamorphosed to some degree with distinct adhesive papillae (Fig. 1). When the larvae of this type were treated with the solution of Nile Blue (1% Nile Blue 1 drop in 10 CC sea-water) for five to seven minutes, then removed

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to the normal sea-water, their tails absorbed completely within 30 minutes (Fig. 2). This result was the same as that of the previous experiment (Hirai, 1962 and 1963).

On the other hand, the other type was the larvae which had the elongated ampullae or rudiments of the roots of the adult. The ampullae were about four to five in number and elongated normally (Fig. 3). The ampullae appeared from the ectodermal area near the adhesive papillae. When the ampullae appeared, the adhesive papillae were not observed in the living material. When the larvae of this type were treated with the solution of Nile Blue, the tails never absorbed and the movement of the tail continued even 18 hours after the treatment (Fig. 4).

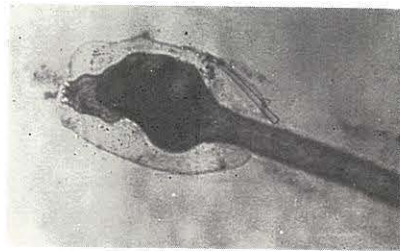


Fig. 1

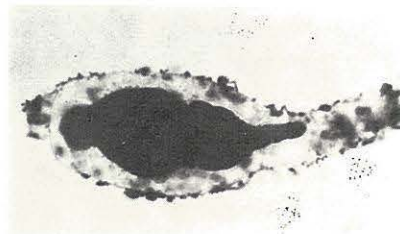


Fig. 2

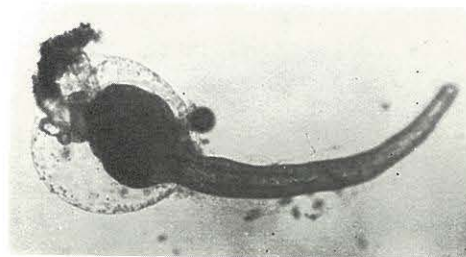


Fig. 3



Fig. 4

Fig. 1. A larva of *Halocynthia roretzi* treated by lower temperature for eight days after hatching. Adhesive papillae and an unabsorbed tail are visible.

Fig. 2. Same larva of Fig. 1 was stimulated by solution of Nile Blue. The tail is absorbed within 30 minutes.

Fig. 3. A larva with elongated ampullae treated by lower temperature for eight days.

Fig. 4. Same larva of Fig. 3 was stimulated by the solution of Nile Blue. Proceeding of metamorphosis is not visible even after 18 hours. End of tail bent behind.

#### CONSIDERATION

In the metamorphosis of the larva of *H. roretzi*, the tail absorption can be disturbed by the treatment with the lower temperature. Among the larvae treated by the lower temperature, those which showed no differentiation of the ampullae

and had distinct adhesive papillae were possible to be awakened again to proceed further differentiation of metamorphosis by the treatment with the solution of Nile Blue (Hirai, 1962 and 1963).

On the other hand, the larvae with the elongated ampullae without the tail absorption were observed and described in this experiment. In those larvae, the adhesive papillae were not observed, and they could not be awakened for further development by the treatment with Nile Blue. From this fact, it is supposed that the ampullae can develop independently of the tail absorption, and that the adhesive papillae disappeared with the differentiation of the ampullae. According to the disappearance of the adhesive papilla, which was supposed as a responder of stimulation for the metamorphosis (Hirai, 1964), the stimulation of Nile Blue will not be accepted naturally for the larvae.

#### SUMMARY

In the larvae of *Halocynthia roretzi* which is treated by lower temperature, a new fact in the metamorphosis was found in the present experiment. This is to say, the ampulla can be differentiated independently of the absorption of the tail. The adhesive papilla disappear with the differentiation of the ampulla. In such larvae with ampulla and with disappeared adhesive papillae, further proceedings of the differentiations of the metamorphosis are disturbed even by the stimulation of the solution of Nile Blue.

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